Proceeding of the 3rd International Conference on Bioscience and Biotechnology, Vol. 3, 2018, pp. 17-27

ISSN 2513-2695 online
DOI: https://doi.org/10.17501/biotech.2018.3103

PRODUCTION OF PROTEIN HYDROLYSATES FROM FISH SKIN FOR DAIRY PRODUCTS

E.I. Titov [1], G.V. Semenov [1], I.I. Ionova [1], I.S. Krasnova [1*] and N.S. Kozlov [1]

[1] *Moscow State University of food production, Russia*

Abstract: The aim of this research was studying the fish protein hydrolysates (FPH) produced from Salmon (*Salmo salar*) skin prepared by different methods. Four methods were used: enzymatic hydrolysis, acid hydrolysis, chemical (with acids or alkalis) and enzymatic hydrolysis with preserving agents upon the heating proposed to produce fish protein hydrolysates (FPH). All methods modified and adapted to optimized researches. All hydrolysates were vacuum freeze-dried and collagen content was determined in them. Depending on the collagen content in the FPH, methods may be ranked as follows: chemical method (with acids or alkali) (15,1%) < enzymatic hydrolysis (26,5%) < acid hydrolysis (30,0%) < enzymatic hydrolysis with preserving agents upon the heating (37,5%). The results showed that FPH prepared using enzymatic hydrolysis with preserving agents upon the heating had a greater amount of protein than FPH prepared using other methods. Citric acid preparation and sesame seeds covered the initial fish odour and off-flavours generated in fish hydrolysates. The sensory analysis of the final product allowed choosing the most rational content of components. Hydrolysis with preserving agents on the heating allowed obtaining collagen hydrolysate in dry form and without fish smell. This FPH may be used as a stabilizer in dairy products.

Keywords: fish protein hydrolysates, hydrolysis, fish odour, dairy products

Introduction

The fish raw material should be treated as a separate category among the sources of collagen. Collagen derived from hydrolysis has a number of advantages over its animal counterpart (Mintz *et al.*, 1991, Yang *et al.*, 2001, Dillow A. K. and Lowman A. M., 2002, Yamamoto *et al.*, 2014). Availability of raw materials is an important factor, the skin of marine and freshwater fish, and the rest raw material from the fisheries is widely used as a source of fish-derived collagen.

Fish collagen has a specific amino acid composition, with a high content of glycine, praline, and hydroxyproline. When ingested, collagen does not completely decompose to amino acids, which, combined with a small molecular weight and particle size, allows it to be absorbed through the walls of the intestine and spread throughout the body and bloodstream. Due to the high degree of identity of the biochemical composition of fish collagen to human collagen (up to 96%), cells can be stimulated to synthesize collagen in articular tissues, bones, skin dermis and other body systems (Bae *et al.*, 2008, Proksch *et al.*, 2014, Hashim *et al.*, 2015, Ramasamy and Ramadhar K, 2015).

Protein hydrolysates of fish-derived collagen have a great value. In the process of decomposition, collagen is broken down into individual peptides which have low molecular weight and as a result, penetrate through cell membranes faster, which allows classification of collagen-containing additives as functional components. During the transition to gluten and gelatine, collagen has the properties of dietary fibre, promoting metabolic processes, and also having a positive effect on the condition and functioning of the beneficial intestinal microflora (Silva *et al.*, 2014). Due to these properties, fish collagen hydrolysates show potential for the use in the composition of dairy products for normalization of microflora, restoration of cartilaginous and connective tissue and at the same time for giving the product the necessary structure (Shori, *et al.*, 2013).

Corresponding Author Email: *ira3891@mail.ru

The limiting factor for an extensive use of fish protein hydrolysate is the presence of a strong fish odour. In this regard, the aim of this study was to make a comparative analysis of various methods of collagen hydrolysis currently used and the choice of the suitable method and optimum conditions for producing collagen hydrolysates for use in dairy technology.

Materials and methods

Skin of salmon fish which is belonging to the salmon family was used. The skin of the fish was washed, ground to a homogeneous state and divided into four parts for conducting hydrolysis using four methods. All methods were modified and adapted for the aims of the research, taking into account the specificity of dairy products.

Enzymatic Hydrolysis

Hydrolysis of fish skin was carried out under the action of proteolytic enzymes. The enzyme preparation: "Protepsin" was used for hydrolysis. The concentration of the enzyme preparation was 1:7 g / kg of raw material. Hydrolysis occurred in cheese whey medium at a temperature of 50 ± 2°C for 3 hours, which resulted in acceptable sensorial parameters of the finished product. Inactivation of the enzyme was performed by heating the solution to the boiling point. The product was then purified, degreased and clarified with a 1% solution of chitosan in 0.1 mol/l hydrochloric acid solution (raw material to chitosan solution weight ratio was 1:0.1). Further, the product was subjected to vacuum freeze-drying (Shironina, 2015).

Acid Hydrolysis

The skin of salmon, with scales and muscle tissue removed, was subjected to peroxide-alkaline treatment for two hours, with a component ratio of 7: 3. After that, the swollen and partially discoloured skin was placed in a 0.5% acetic acid solution for 72 hours. The resulting collagen dispersion was a translucent gel-like substance. It was then sent to freeze drying.

Chemical Hydrolysis (With Acids and Alkalis)

The method of complex processing (with acids and alkalis) of fish raw materials consisted of several successive stages of treatment with inorganic chemicals, during which alkaline and enzymatic hydrolysis occurs. The skin of Salmon scraped from scales and muscle fibre residues were crushed to a size of 2-3 mm and subjected to aqueous extraction at 40-45°C for 45 minutes at a feed- water ratio of 1: 1. The resulting solution was filtered, after which it was sent for treatment to obtain hyaluronic acid. The solid fraction was subjected to bleaching. 3% hydrogen peroxide was mixed with sodium chloride (20 g salt per 1L peroxide) and the raw material was steeped in the resulting solution for 12 hours. This was followed by filtration with separation of the solid fraction, which was placed in a 1.0-1.2% sodium hydroxide solution (raw material-to-solution weight ratio 1: 1) for 24 hours at 20-25°C. Once swelling was complete, the solution was neutralized with a 3% solution of boric acid. The swollen raw material was then proteolyzed with the enzyme «Protepsin» (0.5-0.6% of the solid fraction weight) for 1.5-2.0 hours at 37-40°C. The resulting product was rinsed with cold water and sent to freeze drying.

Hydrothermal Method

This method included the rinsing of raw fish material and heat treatment (80°C) in a cheese whey medium at a ratio of 1: 3 for 60 minutes. This was followed by the separation of the liquid phase, with the solid fraction placed in a clean whey solution and re-treated. The liquid phase was cooled and degreased by centrifugation. The isolation was carried out three times. Then the liquid phases were mixed, cooled and vacuum freeze dried.

Removing fishy Odour

A significant disadvantage of fish collagen hydrolysates is the presence of a specific fishy odour, which can adversely affect the flavour of the finished product. To disguise the specific fish odour, citric acid and sesame seeds were added in a mount of 1 to 5% to the raw material before freezing and freeze-drying

Determination of the Content of Collagen

The content of collagen in the resultant dried samples was determined using a modification of the Neuman and Logan method and expressed in the collagen content as % of the dry matter. The method is based on the isolation of hydroxyproline in the acid hydrolysis of the product sample, carrying out a colour reaction with the products of its oxidation and measuring the intensity of the developing colour. Spectrophotometer «Specord M 40» was used to measure the colour. The obtained data on hydroxyproline were reduced to a standard form for collagen-containing products (% of total protein content), using a special index of 7.63 (for collagen).

Preparation of Yoghurt

Yoghurt was prepared according to the following technology: skimmed milk powder was reconstituted with water at 45°C, then cooled to 10°C and held for 12 hours. The reconstituted skimmed milk was pasteurized at 83-85°C for 15 minutes and cooled to 42°C, then fermented. The composition of the starter included Thermophilic *Streptococcus* and *Lactobacillus bulgaricus*. Yoghurt samples were soured at 41±1°C until curd was obtained. Collagen hydrolysates in the amount of 1, 3 and 5% of the weight of reconstituted milk were introduced into the product in a dry form, before heat treatment. The yoghurt samples were then cooled to 4±2°C, viscosity values were evaluated and sensorial evaluation was determined.

Preparation of Sour Cream

Sour cream was prepared using the thermostatic method. Standardized cream with a fat content of 15% was homogenized at 60-85°C and a homogenization pressure of 12 MPa, then fish hydrolysate (as in yoghurt preparation) was added in a content of 1,3 and 5%, then pasteurized at 86±2°C for 2-10 minutes and cooled to a fermentation temperature of 32±2°C, fermented with a starter composed of *Streptococcus lactis* and thermophilic *Streptococcus*. The fermented cream was stirred, packed and allowed to ripen for 8 hours. The sour cream was allowed to ripen until curd was formed and acidity of at least 65°T was reached, then the sour cream was sent to the refrigerating chamber for cooling to 4±2°C and sour cream ripening.

Determination of the Viscosity of Dairy Products

The viscosity was measured using a low viscosity capillary type viscometer RHEOTEST 2. The shear stress values were measured at a strain rate from 0.167 to145.8 s-1. The temperature was always the same, equal to 20±1°C. The measurement results were mathematically processed in Table Curve® and Microsoft Office Excel using the Ostwald–de Ville model. The controls were yoghurt and sour cream samples prepared using traditional hydrolysate-free technology.

Sensory evaluation of prepared Dairy Products

Sensory evaluation of the products was carried out for the following parameters: colour, taste, smell, consistency and overall acceptance. The control was the traditional yoghurt and sour cream, prepared without fish hydrolysates. The panelists evaluated each attribute on a ten-point scale against traditional hydrolysate-free yoghurt and sour cream. Each panelist received individually about 15-20 ml of the control and other samples.

Statistical analysis

The results are presented as values ± standard deviation (SD). Tukey's test ($P < 0.05$) (Bower, 2013) was used to determine significant deviations between measurements. P-values below 0.05 were considered significant.

Results and Discussion

Hydrolysis is one of the ways to produce protein products from low-value raw materials, which allows obtaining preparations of isolated collagen proteins with high purity, as well as stimulation of the formation of the most important functional and technological properties in the context of the food industry branches, in particular, in the context of dairy products production.

After enzymatic hydrolysis, the resulting hydrolysate had a fibrous structure and a cream-white colour. Particle size was 1-2 mm (Figure 1.1). The hydrolysate formed a gel when adding water. The degree of hydration was 1:4.

Using the hydrothermal method, a fine powder with a colour ranging from white to cream-white with a particle size of up to 500 µm and a slightly fish odour was obtained (Figure 1.2).

The use of combined chemical hydrolysis (with acids and alkalis) allowed obtaining a hydrolysate in the form of a coarse white powder (Figure 1.3), which, when reconstituted with water, turned into a stable translucent dispersion, the degree of hydration was 1:5.

It was not possible to completely eliminate the reagents in the hydrolysate obtained by acid hydrolysis. So, it didn't allow using this hydrolysate as a component in food products. In addition, this hydrolysate cannot be used in dairy products, since it does not dissolve in an aqueous medium.

Figure 1. Hydrolysates after vacuum freeze drying: 1 - hydrolysate obtained by enzymatic hydrolysis; 2 - hydrolysate obtained by hydrothermal method; 3 – hydrolysate obtained by chemical hydrolysis (with acids and alkalis).

The use of citric acid as a traditional means for disguising fish odour proved to be ineffective: no concentration of lemon preparation allowed reaching an acceptable level of disguising of fish odour. At a maximum dosage of 5%, a pronounced lemon smell, in combination with fish odour, produced an even more unpleasant effect. Sesame seeds served as a sorbent for aroma-forming compounds, allowing disguising fish odour. The rational content of added seed was 3% of the raw materials mass. With a greater content of the application, the aroma of sesame began to prevail, which was also extraneous and undesirable from the perspective of the experiment's aims.

Table 1 shows, those samples of hydrolysates after enzymatic and acid hydrolysis contained approximately the same content of hydroxyproline. The minimum content of hydroxyproline was isolated by chemical hydrolysis (with acids and alkalis). The highest content of collagen was found in the hydrolysate produced by the hydrothermal method from fish skin collagen. For this reason, this hydrolysate was selected for further researches.

Table 1 Hydroxyproline Content in Skin and Collagen Content calculated from the hydroxyproline values in the research hydrolysates. Hydroxyproline (OHPro) content in skin (g OHPro/100 g skin), collagen content calculated from the hydroxiproline values (g collagen/100 g hydrolysate)

Method	Hydroxyproline Content in Skin, %	Collagen Content (%)
Enzymatic Hydrolysis	3,47±0,16	26,5±0,03
Acid Hydrolysis	3,93±0,07	30±0,09
Chemical Hydrolysis (With Acids and Alkalis)	1,98±0,11	15,1±0,14
Hydrothermal Method	4,91±0,13	37,5±0,07

Collagen hydrolysate was added to yoghurt and sour cream, since for them one of the most important properties is thickness texture and resistance to flow or motion.

The results of the dependence of the shear stress on the shear rate of yoghurt and sour cream are shown in Figures 2 and Figure 3.

The data shows that for the researched products the force required to break the texture of the samples produced with collagen hydrolysate is higher than that in the control samples. The texture of the obtained hydrolysates, the increase in the content of dry components in dairy products, the binding and distribution of moisture in the texture of the protein gel framework contribute to the increase in the viscosity of the products. The use of collagen hydrolysates with a higher concentration (5%) leads to the production of sour-milk products with a more viscous consistency, while lower concentrations (from 1 to 3%) are advisable for production of drinking yoghurt.

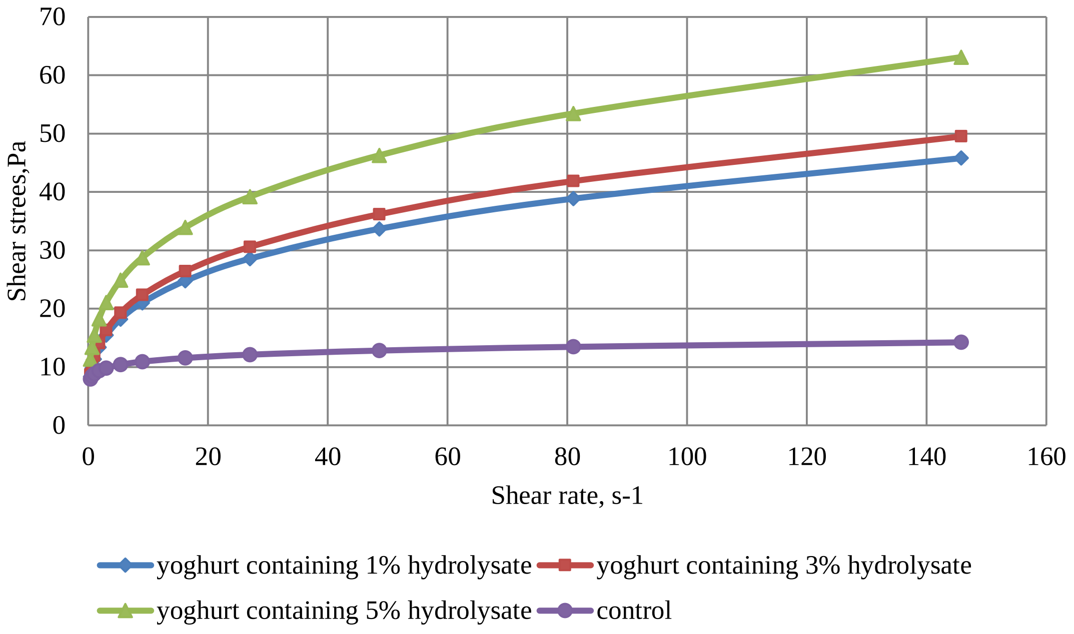

Figure 2. Dependence of shear stress on shear rate of yoghurt. Sample 1 - yoghurt containing 1% collagen hydrolysate, sample 2 - yoghurt containing 3% collagen hydrolysate, sample 3 - yoghurt containing 5% collagen hydrolysate, control - hydrolysate-free yoghurt

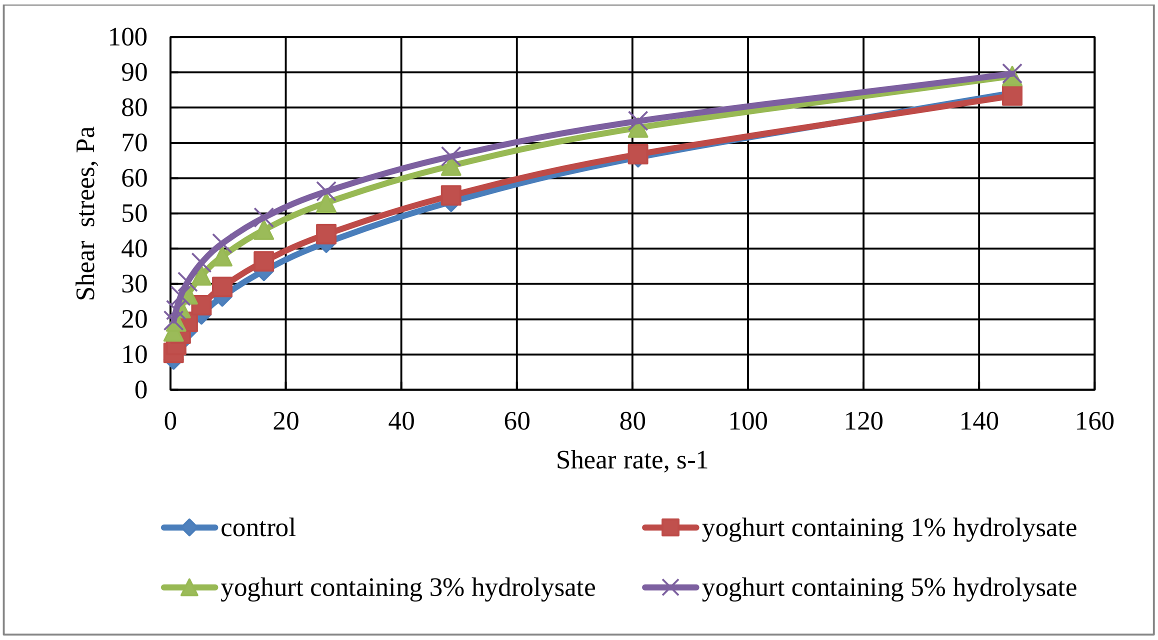

Figure 3. Dependence of shear stress on shear rate of sour cream: Sample 1 - sour cream containing 1% collagen hydrolysate, sample 2 - sour cream containing 3% collagen hydrolysate, sample 3 - sour cream containing 5% collagen hydrolysate, control - hydrolysate-free sour cream.

The sensorial properties of dairy products containing fish hydrolysates proved to be comparable to control samples produced by the traditional technology without hydrolysate. The addition of hydrolysate didn't affect the colour of the product. Also, what is very important, the yoghurt had a rich sour-milk taste without specific fish taste and smell. The panelists noted that the addition of 1 % hydrolysate had virtually no effect on the consistency of the yoghurt. The addition of 3 and 5% collagen hydrolysate led to yoghurt forming a thicker consistency. Panelists were noted in the yoghurt with the addition of 3% fish hydrolysate the best sensorial attributes. Yoghurt containing 5% fish hydrolysate has thicker structure, not characteristic of traditional yoghurt.

Similar data were obtained during the sensorial evaluation of sour cream produced with the addition of collagen hydrolysate. The panelists noted no fish taste and smell in the developed product. Collagen hydrolysate also had an effect on the consistency of sour cream, a thicker consistency was noted in the researched samples, which is confirmed by the data obtained in the researched of viscosity. In terms of the overall acceptance, the panelists gave preference to the sour cream with a content of collagen hydrolysate from 1 to 3%.

The results of the study revealed that the hydrothermal method is the most rational way of producing collagen hydrolysate for use in fermented dairy products. The use of fish hydrolysate in the production of yoghurt and sour cream improves the viscosity of dairy products, their consistency and sensorial properties.

Acknowledgements

This research was financially supported by the Grants №15.8772.2017/БЧ of the Ministry of education and science of the Russian Federation (identification number 15.8772.2017/8.9).

References

Bae, I., Osatomi, K., Yoshida, A., Osako, K., Yamaguchi, A. and Hara, K. 2008, Biochemical properties of acid-soluble collagens extracted from the skins of underutilised fishes. *Food Chemistry*, 108, 49-54.

Bower, J., 2013, *Statistical Methods for food Science*. (Edinburgh: Wiley-Blackwell).

Dillow A. K. and Lowman A. M., 2002, *Biomimetic Materials and Design: Biointerfacial Strategies, Tissue Engineering, and Targeted Drug Delivery* (Mercel Dekker, New York, NY, USA).

Hashim, P., Mohd Ridzwan, M. S., Bakar, J. and Mat Hashim, D., 2015, Collagen in food and beverage industries. *International Food Research Journal*, 22(1), 1-8.

Mintz, G.R., Reinhart, G.M. and Lent, B., 1991, Relationship between collagen hydrolysate molecular weight and peptide substantivity to hair. *Journal of the Society of Cosmetic Chemists*, 42, 35-44.

Proksch, E., Schunck, M., Zague, V., Degwert, J. and Oesser, S., 2014, Oral intake of specific bioactive collagen peptides reduces skin wrinkles and increases dermal matrix synthesis. *Skin Pharmacology and Physiology*, 27(3), 113-119.

Ramasamy S., and Ramadhar K., 2015, A Novel Enzymatic Method for Preparation and Characterization of Collagen Film from Swim Bladder of Fish Rohu (Labeo rohita). *Food and Nutrition Sciences*, 6(15), 1468-1478.

Silva T.H., Moreira-Silva J., Marques A.L.P., Domingues A., Bayon Y. and Reis R. L., 2014, Marine Origin Collagens and Its Potential Applications. *Mar. Drugs*, 12, 5881-5901.

.Shironina, A.Y. 2015. *Improving the Technology of Fish Proteins Proteolysis and the Study of Colloid Chemical Properties of Hydrolysates*. (Murmansk, Russia).

Shori, A.B., Baba, A.S., and Chuah, P.F., 2013, The effects of fish collagen on the proteolysis of milk proteins, ACE inhibitory activity and sensory evaluation of plain- and Allium sativum-yogurt. *J. Taiwan Inst. Chem. Eng*. 9, 1-6

Thorkelsson, G., Slizyte, R., Gildberg, A., Kristinsson, H.G., 2009, Fish proteins and peptide products: processing methods, quality and functional properties, in Marine Functional Food, Edited by J.B. Luten, (Wageningen Academic Publishers), pp.115-140.

Yamamoto, K., Igawa, K., Sugimoto, K., Yoshizawa, Y., Yanagiguchi, K., Ikeda, T., Yamada, S. and Hayashi, Y., 2014, Biological safety of fish (tilapia) collagen. *Biomed. Res. Int*., 1-9.

Yang S.-F., Leong K.-F., Du Z.-H., and Chua C.-K., 2001, The design of scaffolds for use in tissue engineering - part I. Traditional factors, *Tissue Engineering*, 7(6), 679–689.

Appendix

Figure 1. Hydrolysates after vacuum freeze drying: 1 - hydrolysate obtained by enzymatic hydrolysis, 2 - hydrolysate obtained by chemical hydrolysis (with acids and alkalis), 3 - hydrolysate obtained by the method of natural structuring (hydrothermal method)

Table 1 Hydroxyproline Content in Skin and Collagen Content calculated from the hydroxyproline values in the research hydrolysates. Hydroxyproline (OHPro) content in skin (g OHPro/100 g skin), collagen content calculated from the hydroxiproline values (g collagen/100 g hydrolysate)

Method	Hydroxyproline Content in Skin, %	Collagen Content (%)
Enzymatic Hydrolysis	3,47±0,16	26,5±0,03
Acid Hydrolysis	3,93±0,07	30±0,09
Chemical Hydrolysis (With Acids and Alkalis)	1,98±0,11	15,1±0,14
Hydrothermal Method	4,91±0,13	37,5±0,07

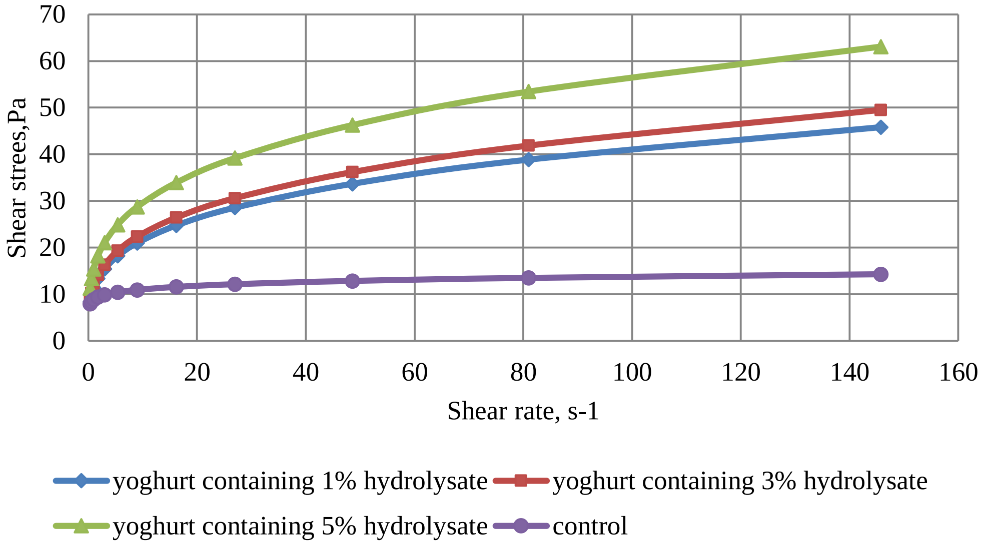

Figure 2. Dependence of shear stress on shear rate of yoghurt. Sample 1 - yoghurt containing 1% fish hydrolysate, sample 2 - yoghurt containing 3% fish hydrolysate, sample 3 - yoghurt containing 5% fish hydrolysate, control - hydrolysate-free yoghurt

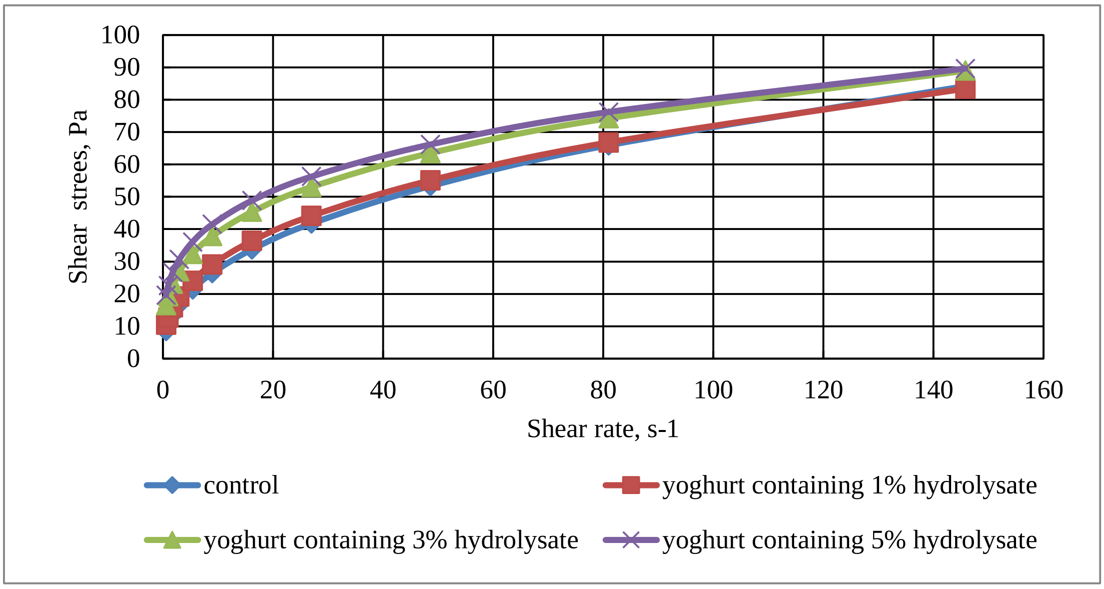

Figure 3. Dependence of shear stress on shear rate of sour cream: Sample 1 - sour cream containing 1% fish hydrolysate; sample 2 - sour cream containing 3% fish hydrolysate; sample 3 - sour cream containing 5% fish hydrolysate, control - hydrolysate-free sour cream.